IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of)
:
Yuki Katayama, et al.)
: Group Art Unit: 1657
Serial No. 10/531,315)
: Examiner: Lisa J. Hobbs
Filed: April 13, 2005)
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For: METHOD FOR QUANTITATIVELY DETERMINING CHOLESTEROL IN HIGH-DENSITY LIPOPROTEIN AND REAGENTS THEREFOR
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DECLARATION

The Honorable Commissioner of
Patents and Trademarks
Washington, D.C. 20231

Sir:

I, Yuki Katayama of 905 SURPASS MISHIMAHONCHO, 1-10, Honcho, Mishima-shi, Sizuoka, Japan do declare as follows:

I finished my bachelor course of Agricultural Sciences, Faculty of Agriculture, Okayama University in March, 1995, and I was given the degree of B.A. I finished my master course at Graduate School of Agriculture, Okayama University in March, 1997, and I was given the degree of M.A.

Since April, 1997, I have been employed by KYOWA MEDEX CO., LTD.

Since April, 1997, I have been engaged in the research on development of diagnostic reagent kits, mainly related to lipids (e.g. HDL cholesterol).

In the Experiments shown below, I used the following reagents and enzymes.

HEPES (manufactured by BDH Laboratory), EMSE (manufactured by Daito Chemix Corporation), sodium dextran

sulfate (molecular weight: 500,000) (manufactured by Pharmacia), bovine serum albumin (BSA; manufactured by Oriental Yeast), 4-aminoantipyrine (manufactured by Saikyo Kasei), Peroxidase (manufactured by Toyobo), LPL6 (cholesterol esterase; manufactured by Amano enzyme), COO322 (cholesterol oxidase; manufactured by Toyobo); Nymeen L207 (polyoxyethylene dodecylamine; manufactured by NOF), Newcol OD420 (polyoxyethylene octadecylamine; manufactured by Nippon Nyukazai), Emulgen 120 (polyoxyethylene lauryl ether; manufactured by Kao), Emulgen 220 (polyoxyethylene cetyl ether; manufactured by Kao), Emulgen 320P (polyoxyethylene stearyl ether; manufactured by Kao), Emulgen 420 (polyoxyethylene oleyl ether; manufactured by Kao), Emanon 1112 (polyoxyethylene monolaurate; manufactured by Kao), Emanon 3115 (polyoxyethylene monostearate; manufactured by Kao).

Kits for quantitatively determining HDL cholesterol

Kits for quantitatively determining HDL cholesterol comprising the following first and second reagents were prepared. Table 1 shows the list of Detergent in the First reagent of each of the Kits.

First reagent

HEPES (pH7.5)	10 mmol/L
EMSE	0.3 g/L
sodium dextran sulfate (molecular weight: 500,000)	1.0 g/L
BSA	2.0 g/L
Peroxidase	10 kU/L
Detergent	0.07 g/L

Second reagent

HEPES (pH7.0)	10 mmol/L
4-Aminoantipyrine	0.3 g/L
Peroxidase	20 kU/L
LPL6	0.05 kU/L
COO322	3.0 kU/L

Table 1

Kits	Detergent	
A1	Nymeen L207	polyoxyethylene dodecylamine
A2	Newcol OD420	polyoxyethylene octadecylamine
a1	Emulgen 120	polyoxyethylene lauryl ether
a2	Emulgen 220	polyoxyethylene cetyl ether
a3	Emulgen 320P	polyoxyethylene stearyl ether
a4	Emulgen 420	polyoxyethylene oleyl ether
a5	Emanon 1112	polyoxyethylene monolaurate
a6	Emanon 3115	polyoxyethylene monostearate
b	none	

Of the detergents shown above, polyoxyethylene lauryl ether, polyoxyethylene cetyl ether, polyoxyethylene stearyl ether, polyoxyethylene oleyl ether, polyoxyethylene monolaurate and polyoxyethylene monostearate are disclosed in Miyauchi et al..

Quantitative determination of HDL cholesterol

Using sera obtained from patients suffering from M proteinemia (abbreviated as "M proteinemia serum sample") as a sample and the kits shown above as a kit, concentration of HDL cholesterol in each of M proteinemia sera samples was measured on Hitachi 7170 autoanalyzer.

(1) Preparation of calibration curve

A calibration curve showing the relation between HDL cholesterol concentration and "absorbance" was prepared by the measurement on Hitachi 7170 autoanalyzer using a physiological brine (HDL cholesterol concentration: 0.0 mg/dL) and serum (HDL cholesterol concentration: 60.0 mg/dL) as standard solutions.

"Absorbance" used herein means a value obtained by subtracting E1 from E2 on the basis of the two absorbances (E1 and E2) measured in the following reaction.

A standard solution (3 μL) and the first reagent (0.24 mL) were added to a reaction cell and the mixture was heated at 37°C for 5 minutes. After measurement of absorbance (E1) of the reaction mixture at a main wavelength of 600 nm and a sub-wavelength of 700 nm, the second reagent (0.08 mL) was added to the reaction mixture and the mixture was heated at 37°C for 5 minutes. Absorbance (E2) of the last reaction mixture was measured at a main wavelength of 600 nm and a sub-wavelength of 700 nm.

(2) Calculation of "absorbance" for an M proteinemia serum sample by the reaction of the sample with the Kit

The same method as in the calculation of "absorbance" in (1) was carried out except that M proteinemia sera samples were used instead of the standard solution used in the preparation of a calibration curve in (1) whereupon "absorbance" for the sample was calculated.

(3) Determination of HDL cholesterol concentration in the M proteinemia serum sample

HDL cholesterol concentration in each of the M proteinemia sera samples was determined by correlating the "absorbance" calculated in (2) and the calibration curve prepared in (1).

In the meanwhile, HDL cholesterol concentration in each of the M proteinemia sera samples was determined according to a DCM (a Designated Comparison method) mentioned in Clinical Chemistry, vol. 45, No.10, p. 1803-1812 (1999), and the results are shown in Table 2.

Table 2

Samples	Measurements (mg/dL)									
	DCM**	Kit								
		A1	A2	a1	a2	a3	a4	a5	a6	b
Sample A*	72.2	73.4	76.4	90.6	83.6	86.3	81.0	92.8	92.3	95.3
		(1.7)	(5.8)	(25.5)	(15.8)	(19.5)	(12.2)	(28.5)	(27.8)	(32.0)
Sample B*	65.5	66.8	67.6	82.6	72.1	73.8	71.9	103.7	98.3	109.8
		(2.0)	(3.2)	(26.1)	(10.1)	(12.7)	(9.8)	(58.3)	(50.1)	(67.6)
Sample C*	74.9	73.7	78.4	85.5	62.5	58.3	68.6	83.7	85.2	86.1
		(-1.6)	(4.7)	(14.2)	(-16.6)	(-22.2)	(-8.4)	(11.7)	(13.8)	(15.0)

*Samples A~C: M proteinemia sera samples

**DCM: a comparable method

The values described in parentheses in each Kit column of Table 2 represent difference percentage (%) calculated by formula (I).

difference percentage (%) = $(A_{Kit} - A_{DCM})/A_{DCM} * 100$ (I)

In formula (I), A_{Kit} is a value determined by the measurement using each of the Kits, and A_{DCM} is a value determined by DCM. The nearer the difference percentage is 0%, the more correct value the measurement using the Kit gives.

Apparent from Table 2, the measurements using the Kit A1 comprising polyoxyethylene dodecylamine and the Kit A2 comprising polyoxyethylene octadecylamine of the present invention give difference percentage less than 10%, while the measurements using the Kits a1~a6 and b give difference percentage of 10~70%, which means that correct measurements of HDL cholesterol in M proteinemia sera samples can not be carried out using the Kits a1~a6 and b.

Thus, it proves that the measurements using the Kits of present invention give a correct measurement of HDL cholesterol even in M proteinemia sera samples.

The undersigned declarant declares further that all statements made herein of his knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Executed this 11th day of December, 2009.

Yuki Katayama

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